The Lister Institute of Preventive Medicine.

Chairman of the Governing Body:
SIR HENRY H. DALE, O.M., G.B.E., M.D., F.R.C.P., F.R.S.
Hon. Treasurer:
THE RT. HON. VISCOUNT WAVERLEY, P.C., G.C.B., G.C.S.I., G.C.I.E., F.R.S.
Director:
PROFESSOR A. A. MILES, C.B.E., M.D., F.R.C.P.

CHELSEA BRIDGE ROAD,
LONDON, S.W.1.
Telegrams: "Bacteriology, Knights, London."
Telephone: SLOANE 2181.

3rd.February 1955.

Professor J.Lederberg,
Department of Genetics,
University of Wisconsin,
Madison,
Wis, U.S.A.

Dear Josh,

Thanks for your letters of Jan.5th. & 26th.

(1) To get rid of a few other things first, before getting down to draft etc. First as to Helen B. Thanks for the details about her. I have written to her, more or less on the lines indicated by your report, and hope that we may get something fixed up.

(2) As to terminology. I am not happy with uni-linear, but I do not consider any of your amendments much better. As to "catenate", I suppose you base the term on the idea of individuals of successive generations as the links in the chain. There seems to me an element of ambiguity in the chain-idea, since, especially after reading Jenning's paper, one may envisage the "chain" as that composed of all the members of a clone united end to end. I don't like "primogenitive" as I do not think there is any evidence of a regular polarity (cf.Jennings). And I doubt if "monochotomous" is correct, as "chotomy" means by derivation "cutting" or "splitting" and one can hardly split into one.

(3) If what Jennings and others say about behaviour of contractile vacuoles of Paramoecium is correct, then these are "inherited uni-linearly" as a normal phenomenon. It seems to me that it might be better to try and think of a term to indicate the absence of splitting (multiplication or division) of the particle, and if possible also its (relative) permanence rather than to bring in the more restricted notion of its linear or catenary inheritance. Logically one should speak of an "atomic" particle, but this term is perhaps too laden with the wrong connotations, (popular and scientific). "Achotomous" might be all right. "Quantal" has the right scientific connotation, but little justification by derivation. There must also be some good botanical terms meaning non-branching which we should consider. This needs further thought.

(4) I have to thank you for the J.reference. I am most impressed by its excellence. What a pity he did not coin a word, and so save us all this discussion. (It is a pity Beale did not refer to it in his monograph but I gather it is very incomplete on everything before Sonneborn) You say there are "a number" of analogies: I would be glad of references if possible for I am quite at sea in the literature apart from bacteriology.

(5) I am glad we agree that mono-catenate inheritance of MCP is sufficiently proved: your 59th. generation case should convince anyone. A propos this, I mentioned in an earlier letter some experiments of Quadling, (part of his program for thesis on "genetic and environmental control of motility etc", (and for this reason not available for inclusion in present paper, and not for general circulation as yet). He has been investigating O strains which produce deep colonies spontaneously, and has been able to show by manipulation that there is a very low incidence of motile cells, and that these on isolation transmit motility in oligo-catenate fashion (Maximum so far 5). This is all as one would expect, but some complications from temperature affects etc.

There are several obvious extensions which he will look into in due course, if all goes well, e.g. inheritance after transfer to medium which does not permit further synthesis of MCP. Even though we do not refer to this, I think it still further strengthens our case for "atomic" inheritance of MCP, and indicates it occurs quite independently of any genetic manipulation from without.

(6) As evidence for "atomic" (formed or "aschizoid"?) inheritance of MCP the cases of sibs of transparent cells is perhaps easier to present than that of sibs of E cells. But I have no pedigrees in which the mono-catenate cells (presumed) were re-isolated from the sib clone. You mentioned one such pedigree; could you , when convenient, send me the pedigree ? (for inclusion in draft if we do it as joint paper).

(7) As to draft. I confess I am somewhat disappointed you do not find the evidence for two orders of atomic inheritance convincing as it stands, for I was beginning to feel that I had laboured the point too much. I remain of the opinion that the argument in the paper together with the long pedigrees, is sufficient; but I agree in principle that one cannot be too thorough, so shall discuss your points one by one.

(8) Para 2 and 3 of your letter of Jan.26. I think that my data on 541 establish the unequal distribution of numbers of motile progeny amongst sibs, unequivocally, in cases where total number is greater than 10. I am inclined to attribute your less striking results with 543 to high incidence of trail-endings, either by loss or destruction of "gene", or possibly death of E cell.

(9) As to possible unequal distribution amongst progeny of oligo-catenates. I have also a few instances of distributions like your 7:1:1:0. But I incline to regard these as "short trails", the cell producing the 7 having been in reality an E cell, but having ceased to be so (or died) before it could produce more than 7 (x efficiency of detection factor) mcp. The conclusive argument for non-random distribution can only be where the parent cell is known not to have been an E cell; in fact, only if non-random distribution found in each of two (or more) sub-clones. I have gone through my pedigrees again, and find no such definite evidence. Admittedly the data are not as extensive as one would like nor of the optimum kind; but I have on quite a number of occasions split up the clone produced by a sib of an E cell, and the distributions don't look non-random. I don't think a formal analysis worth while, partly because of the bitty nature of the data and partly because of the difficulty of allowing for the "detection factor". Taking the sib of the E cell as generation 1, I have one instance of one of its descendents of the 4th. generation producing 2 motiles, and one of a 3rd.generation descendent producing 3. These are about what one would expect if the 1st.generation cell contained c 15 mcp and the detection factor was about 1/3. Had there been any gross non-randomness I think it would have been detectable.(Minor non-randomness might result from a slight effect of polarity, or from unequal cell division, and so would not weaken theory).

(10) As to Birset, don't take me too seriously. His evidence for "growing points" struck me as so weak as to be non-existent (though presented with his usual pugnacious dogmaticness when he read a paper on it). I hope you will press on with TZ method some time. I see no theoretical evidence for doubting polarity, just no good evidence for it so far.

(11) Your para 7.* My micro-manip. transfers (in draft and a few others) indicate that c 20% of early motile cells can initiate trail in gel-agar. If as suggested here and in para 16 this represents a probability of 0.2/x that a mono-catenate cell will indicate a trail (when placed on surface of agar) with a mean of X mono-catenates per early motile, then one would expect that $(0.2)^2$ initially would initiate two trails, i.e. that 0.2 of all trails would be "twin-trails". But I saw no definite twin trails when plating diluted 541 suspensions on gelatin agar, and I think I would have detected a proportion as low as 0.1.

see post script

(12) Your para (8). If you mean what I think you mean, you have misunderstood what I did. I caught a lot of early motiles, put them all together in a pool in chamber, and transferred this en masse to a bottle of broth. From this I took out, blindly, drops to gel agar. I therefore don't know the number of motiles per drop, but can infer it from Po ; I see no reason to suppose distribution non-random in the bottle. All cells were motile when picked up; I don't know what proportion viable. I hope this clears up this point, but am not quite sure what you are driving at.

(13) I can see no way of proving directly that polycatenates form trails as the tests for no. of motiles amd for trail-forming ability are mutually exclusive. On the other hand one could of course transfer proved mono-& -catenates to gel-agar, though it would be tedious. As you get "virtually no trails" it is evidently not worth doing with 543. If you think it needs doing, why not try 541?

(14) Your para 10. What was the phenol expt? You did not mention it before.

(15) Your para 12. I have considered this, but avoided mentioning in draft because there is an unexplained anomaly in my data. Viz. trail:swarm ratio on plates is higher than E-cell:motile-clone ratio in experiments in which motiles caught early. I suspect that the lag before development of motility may be longer in case of transformation. My best data for ratio of clones:E-cells:oligos are as follows:-

Expt.1. 57 early motiles. 6 died, 10 gave non-motiles only, 31 gave from 1 to 10 motiles, 11 gave 20-60 motiles. No clones.

Expt.2. 60 early motiles, 1 died, 1 gave non-motiles only, 43 gave from 1 to 18 motiles (mean 3.7; median 3+), 13 gave from 19 to 75 motiles, 2 gave clones, 1 was diagnosed early as probable E and transferred to agar (no trails).

(On going over these data again, I would now consider all the "intermediate" cells, i.e. more than 8 and less than 15 motiles) as probably E cells with early loss of gene. There were 3 such cells in expt.2, producing c 9, 10 and 12 motiles; the distributions as far as followed were:- (3; at least 6)
(5;1), (3;1)
(8; 3;1).

The non-random-looking distribution of the last one fits in with diagnosis of short-lived E cell.

In macro-expts., the trail:swarm ratio is nearer 2:1. This may be because "short trails" are diagnosable as E cells but not detected as trails, or for reason suggested above.

(16) Your para 14. I think the incidence you quote was per treated cell, not per phage. I have done no quantitation on the latter. I have had as many as 3.1×10^{-4} trails per surviving treated cell (survival near 100%) and (about) half this yield of swarms in same experiment.

(17) Your para 15. I assume these to be, probably, sibs of E cells, the synthesis of mcp's having started some time before motility results. Motiles are first detectable in any numbers, under my conditions, after about 2 hours at 37°. In the expts. cited above, in which poly:oligo ratio was about 3:1, the increase in viable count at time of setting up drops was about x 5, which fits in well enough with notion of

3:1 (1;3½)

initiation of mcp very soon after mixing, and distribution during lag before they became effective. (Some of Q's expts. on environmentally induced changes also suggest the possibility of some such lag). This point should, I now think , be briefly discussed in the paper.

(18) Your para 16. I think this can be rejected for reasons stated in para 11 above. It could be disproved (on 541 at least) by comparing yield of trails from early motiles transferred to gel agar with that of mono-catenates from pedigreed cells. It leaves bi-modal distribution (on log scale) of numbers of motil progeny unaccounted for.
of early motiles

(19) Your para 17. In one of my pedigrees a cell of the estimated 22nd. generation produced estimated 100 motile progeny, its ancestor of the c 13th. generation c 150. Some of all other cells of 13th. generation produced c 50. Does this not exclude the hypothesis stated, unless you put it in the form that the original cell has a bundle of, here, 200 mcp's, from which a few only fray off at each generation? In the latter form the theory is experimentally indistinguishable from mine; one could only go on and hope to isolate an E cell after so many generations that the "bundle" would have to be absurdly large. In the pedigree cited, as there were c 200 motiles seen, it must be of 200 units; and as the "detection factor" comes in here too, probably more than 600 really. I do not myself feel that further micro-manipulation pedigrees are really needed to test this. The trails which continue to lengthen for over 24 hours indicate that an E cell may persist as such for 70 generations or more (This I do not put forward as a repetition of your hypothesis).

I agree the E "particle" could be an enzyme system, but this is to postulate an unnecessary entity, since "gene" transfer required for effective transformation.

(20) Your para 19. I agree, and have mentioned possible (certain really) intermediates between "gas" of E cell and the mcp. I agree there may be more than 2 classes, and that these may or may not be replicate; but I don't think it possible to escape 2 classes of non-replicating particle by anything but highly implausible hypotheses. (I have not yet looked up your refs. here).

(21) Tape would be fine. I have no means of playing except chez Andy; he might be a bit surprised if told it was confidential, but I don't suppose you are really going to say anything too terrible.

(22) Publication. As to preliminary publication, I am agreeable in principle. However, I suggest Nature as journal rather than P.N.A.S., since it is I suspect more widely seen (outside U.S.) and since P.N.A.S. states its function as ".....publication of work of American scientists" which would make it a bit inappropriate in this case. As to content, would it not be very difficult to present the uni-catenate data without more or less committing ourselves on the E cell bit also? (Not that I am averse to this but I guess you still are). Would you care to do a skeleton draft? to show what you have in mind?

(23) Why the objection to a microbiological journal ? The J.G.M. is I suppose fairly readily available to geneticists (or such of them as are interested in this kind of thing). The genetic journals are not so readily available to many bacteriologists, some of whom like to follow this kind of work.

(24) The Genetical Society are having a meeting soon. I propose to read a paper on this, title not yet decided. As time is short and we are not yet in complete accord, I will present my work only but propose to mention, while speaking, your parallel and concordant results. I take it this is agreeable to you.

(25) How about a note for the M.G.B.? I think it would be quite interesting to their readers, and as it is not publication, we need not be too cautious. It looks as though there will be some delay before proper paper, so I think it would serve a useful purpose.

(26) Sorry this is so long; but I think most points covered. Attempt to electron microscope mono-catenate cells again held up; microscopist after keeping last lot 2 weeks without examining has now gone for trip to U.S. for a month. Rumour has it you are off to Palestine for a sabbatical year some time, I don't know if right; if so I hope you manage to include London en route.

Yours sincerely,

B.Stocker.

On reading this over ~~before~~ after typing, I feel perhaps I have not really dealt adequately with your two 'devilish' points. However I think I will send this off as it is, you must disregard any bits which are nonsense or too dogmatic. I will think over & write again, ~~shortly~~ soon I hope: it might be best if I re-drafted the missing section on discussion of 'atomic' gene-like particle to try to cover them.

Yrs Bruce.

Glad house is satisfactory. It sounds nice. My flat is improving considerably now I have more furniture. Love to Esther, tell her I expect a newsy letter soon.